bio-protocol Preprint

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Cell clustering and classification

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Updated date: Aug 27, 2021

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An abbreviated version of this protocol was published in eLIFE in Feb 2019

Specification of diverse cell types during early neurogenesis of the mouse cerebellum

DOI: 10.7554/eLife.42388

Detailed protocol

GENERATION OF SINGLE CELL SUSPENSION FOR SCRNASEQ ANALYSIS (James Li Lab)

I. **Preparation: filter buffer1 and resuspension buffer with 0.22um filter.**
   a. Prepare 10 ml cell Resuspension buffer(RB) as following: DMEM/F12 w/o phenol red (9.51 ml) + 2% FBS(200µl) + 2mM EDTA (40µl of 0.5M) + 25mM Hepes (250µl of 1M)
   b. Prepare 50ml Buffer 1 = PBS with 1%BSA(1.43ml of 35%) + 2mM EDTA (200µl of 0.5M)
   c. Prepare Papain (3 tubes): add 400µl of HBSS to 50µl 10x papain, warm up in 37˚C water bath.
   d. Precool solutions on ice: HBSS, Buffer I (3 tubes-- 1.5 ml in a 5ml Eppendorf tube), and Resuspension Buffer.
   e. Precool Bench top Centrifuges to 4˚C.

I. **Cell dissociation**
   1. Dissect embryos in ice-cold HBSS; dissect out cerebellum. Rinse four times in HBSS in a 24-well plate.
   2. Cut cerebellum into small pieces (about 1 mm in dimension) with fine scissors in a drop of HBSS (about 50µl) in a dish on an ice tray under the microscope.
   3. Add 10µl of DNAseI (1mg/ml) to 450µl prewarmed papain.
   4. Transfer the tissues to papain + DNAseI solution.
   5. Incubate at 37˚C for 30 minutes with rotation.
   6. Using P1000 tip to pipet up and down, gradually reduce the distance from the bottom of the tube.
   7. Use glass pipet with gradually smaller opening to gently pipet tissues up and down to dissociate cells.
   8. Put 70-µm cell strainer on top of the 5-ml Eppendorf tube containing 1.5 ml of Buffer 1. Pass entire mixture through the strainer into the tube – gently press the strainer and slowly pipetting the liquid.
   9. Wash cell strainer twice with 500µl buffer 1 each and add it to the 5ml tube. The total volume is 3ml now.
   10. After filtration, pellet cells in the 5ml Eppendorf tube at 300 x *g* for 10 minutes at 4˚C.
   11. Remove the supernatant; gently flick the tube to dislodge the pellet.
   12. If red cell was observed in pellet, do ASK lysis to remove blood cell.
   13. Resuspend the pellet in Resuspension Buffer (RB): add 100 µl RB buffer
   14. Take out 1 tube of 2x LiveDeath Assay Mix from -20C. Add equal amount of PBS. Then add 10µl of cell suspension to mix with 10 µl of 1x LiveDeath Assay Mix; incubate at RT for 10 minutes.
   15. Add 10 µl mixture to a hemacytometor chamber and image.

Reagents:

Papain (Worthington Biochemical Corporation, #LK003176): Add 1 ml HBSS with Ca+ and Mg+) to 1 vial of Papain; Aliquot 50 µl/tube and store at -20˚C.
Buffer1 (1%BSA and 2mM EDTA in PBS): Add 0.2 ml of 0.5M EDTA and 0.5g BSA to 50 ml of PBS; filter with 0.22 µm filter.
HBSS (Hanks Balanced salt solution, life technologies Cat# 14025-092)
LiveDeath Assay Mix (2x): mix 10µl EthD-1 (2mM, Invitrogen), 5µl Calcein (4mM, Invitrogen), and 2.5µl Hoechst (16.2 mM, Invitrogen) to a final volume of PBS; aliquot 25µl/tube and store at -20˚C.

MACS Tissue Storage Solution (Miltenyi Biotec, Catalog #130-100-008)
RPMI 1640 Medium, no phenol red (Thermofhisher, Catalog #11835030)
Resuspension Buffer (Lebovitz L15 medium with 2% FBS, 25mM HEPES, 2mM EDTA)
Lebovitz L15 medium, no phenol red (Gibco, Catalog #21-083-027)

Disposables:

Cell strainer size 100 µm (Corning, Catalog #431752)
5ml Eppendorf Tubes (Fisher, Catalog #05-412-581)
Wide Bore Tips

How to cite:(Readers should cite both the Bio-protocol preprint and the original research article where this protocol was used)

1. Li, j. (2021). Cell clustering and classification. Bio-protocol Preprint. bio-protocol.org/prep1351.
2. Wizeman, J. W., Guo, Q., Wilion, E. M. and Li, J. Y.(2019). Specification of diverse cell types during early neurogenesis of the mouse cerebellum. eLIFE. DOI: 10.7554/eLife.42388